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# Sustainable Fertilizer Strategies for *Vaccinium corymbosum* x *V. angustifolium* under Abandoned Peatland Conditions

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**Abstract:** Revegetating abandoned peatlands plays an important role in reducing the CO<sub>2</sub> footprint. One possibility for carbon reduction is cultivating blueberries as calcifuge plants in acidic peat soil. The aim of the experiment was to find out the effect of different fertilizers on half-highbush blueberry cultivar ‘Northblue’ growth and biochemical parameters in peatland conditions. The experiment was carried out in 2011–2015 with four organic and one mineral fertilizer, where three were composted chicken manure- and one maltose-based organic fertilizer. The soil of the experimental area belongs to the soil subgroup *Fibri-Dystric Histosol* with the peat layer 1.0–1.5 m deep. Organic fertilizer 4–1–2, which contained seaweed but had low phosphorus and potassium content, resulted in high yields in 2011 and 2013, with similar vegetative growth and comparable biochemical parameters as mineral fertilizer 6–14–23. The principal component analysis showed that the experimental year was more important in determining fruit parameters than the fertilizer type. However, our results indicated that the organic fertilizers are alternatives to mineral fertilizer for organic production.

**Keywords:** anthocyanin; total phenol content; growth parameters; peatland revegetation; organic fertilizers; yield

## 1. Introduction

In the European horticulture industry, Estonia is a remarkable peat exporter [1]. Peat is mostly used as an organic amendment for soil improvement and as a substrate for ornamental plants and vegetable production, but is also used as fuel for household heating. Since abandoned peatland constitutes substantial CO<sub>2</sub> emissions—ca. 8 million metric tons annually, which is more than all Estonian cars and trucks produce combined [2,3]—restoring or revegetating these areas is vital for sustainable environmental management. In Estonia, peatlands with a depth greater than 30 cm cover an area of about 915,000 ha [4], of which about 5000 ha are abandoned peat mining areas, while on another 11,000 ha, mining is still in progress [2]. About 25% of European peatlands are located in the Baltic Sea basin, making up at least 14% of the basin area [5]. Many of these (41%) are located in the northern sub-basin—in the Bothnian Bay area, which is the northern part of Baltic Sea, where Estonia situates. After Finland and Sweden, Estonia is third in Europe in terms of area of peatland [6].

The content of available mineral elements in peat soil is small, because organic matter mineralization after decomposition is a time-consuming process [7,8]. Therefore, plant growth on peat soil is highly fertilizer dependent [9,10]. Growing calcifuge plants with fertilizers on abandoned peatlands is one of the options to reduce carbon emission. It was concluded from an earlier study

in Estonia that blueberry (*Vaccinium* spp.) cultivation on peatlands is economically profitable [10]. The peculiarity of Estonia is that most of the blueberry cultivation follows organic farming principles, as diseases and pests have not yet caused economically important yield losses, thus making organic technologies easily adoptable. In addition, it has been stated that the blueberry fields located on peatlands have lower disease indices [11]. However, there is little information about appropriate organic fertilizers for half-highbush blueberry cultivation on peat soil.

The suitable blueberry species for growing under the northern climatic conditions is a half-highbush blueberry *Vaccinium corymbosum* × *V. angustifolium* [12]. Half-highbush blueberry is an interspecific hybrid of lowbush and highbush (*V. corymbosum* L.) blueberries that has inherited the ability to survive the harsh winters of northern areas [13]. However, winter damage, especially fluctuating temperatures in January and February, is among the major causes of blueberry yield loss [12]. A previous study showed that half-highbush cultivar (cv.) ‘Northblue’ is winter-hardy and performs well under cold climatic conditions. This cultivar has big berries, but contains lower amounts of anthocyanins and other polyphenolic compounds compared to commonly cultivated half-highbush blueberry cv. ‘Northcountry’ [14]. Anthocyanins are bioactive flavonoid compounds that are beneficial against many chronic diseases, and therefore blueberry is one of the fruits that is popular for its taste and richness in anthocyanins [15]. These compounds are blue, red, or purple pigments that are found in plants, especially flowers, fruits, and tubers. In acidic conditions, anthocyanin appears as a red pigment, while a blue pigment occurs in alkaline conditions. Although the polyphenolic compound content is species-specific, the weather conditions during the vegetative period also have an important effect on biochemical and growth parameters [16]. Previous studies of blueberries on peat and mineral soils have shown that yield and other plant parameters are strongly related to weather conditions, i.e., temperatures, precipitation and sunshine hours [9,17].

Since blueberry forms symbiosis with ericoid mycorrhizal fungi, which decompose the organic matter in the soil [18,19], organic amendments, i.e., organic fertilizers, are important for the soil microorganism and mycorrhizal symbiosis. Blueberry plants need acidic soil, of pH<sub>KCl</sub> 4.0–5.5, which is also rich in organic matter [20]. Previous studies have shown that blueberries grow well in highly acidic peat soil pH<sub>KCl</sub> > 4.0 without the need of liming [17]. Growing blueberries in peat soil provides better nutrition supply compared to mineral soil, resulting improved growth and higher yield [12]. An experiment with different half-highbush blueberry cultivars evaluated the effect of cultural practices on mycorrhizal colonization and stated that the mycorrhizal colonization was higher on the roots of half-highbush cv. ‘Northblue’, with a positive correlation between mycorrhizal colonization and growth and yield [21]. When managed organically, research with a number of crops suggested a consistent reduction in soil-borne diseases [18], increased defense mechanisms of plants such as antioxidant production [22], and an increase in both microorganism diversity and biological activity in the soil [23]. It was confirmed in a previous study that the organic fertilizer increased the soil biota activity, mycorrhizal colonization, and leaf antioxidant content relative to conventional N source, and improved tolerance to soil pathogens [19]. Experiments conducted in peatland have shown that the lowbush blueberry cultivation could be an option for the vegetation restoration of abandoned peatlands as plant cover provides a suitable habitat for various arthropods [24].

Revegetating abandoned peatlands with blueberries could reduce the negative impacts of these environmentally sensitive areas, and therefore, more information on fertilizer strategies needs to be gathered. The hypothesis was that organic fertilizers are suitable for organic production, have a positive effect on the vegetative and biochemical parameters of half-highbush blueberry cv. ‘Northblue’, and are feasible alternatives to mineral fertilizer on peatlands after intensive peat production. The aim of the study was to determine the effect of different fertilizers on half-highbush blueberry cv. ‘Northblue’ plant growth and fruit biochemical parameters in peatland conditions.

## 2. Materials and Methods

### 2.1. Site Description

The experiment was carried out on an abandoned peat field in South Estonia in Tartu county (58° 23' N, 26° 31' E '7'45', H: 33 m). Data were collected in 2011–2013 and in 2015. In 2014, the blueberry plants had severe frost damage, and due to lack of yield, the biochemical analysis were not taken and are not presented in this study.

The experiment was established in the spring of 2006 with one-year-old half-highbush blueberry (*V. corymbosum* × *V. angustifolium*) cv. 'Northblue'. Plants were propagated with hardwood cuttings. The experiment was a randomized complete block design with three replicates with 10 plants per plot, with plant spacing of 1.0 × 1.5 m. Weeding was maintained manually and pesticides were not used. Fertilization was carried out every year once a year, at the beginning of May, which is also the beginning of the growing season in Estonia. The fertilization rate with organic fertilizers was 70 kg/ha N, an additional 50 kg/ha N was given with feather meal 14–1–0. The total N fertilization rate in a year was 120 kg/ha in each fertilizer treatment. All the fertilizers, organic and mineral, used in the experiment were granulated. Fertilizers were selected based on their composition suitability for blueberry cultivation.

One mineral and four organic fertilizers were used in the experiment:

- Min: mineral fertilizer 6–14–23 (plus Mg 3%, S 11%, B 0.05%, Cu 0.1%, Fe 0.1%, Mn 0.7%, Mo 0.01%, Zn 0.01%). Fertilizer 6–14–23 has been commonly used in conventional berry production in Estonia. Mineral fertilizer was considered as the control.
- Org 1: Organic fertilizer 3–1–7 contains mainly composted chicken manure and vinasse extract (9%, potassium rich by-product of the sugar industry) and molasses.
- Org 2: Organic fertilizer 4–1–2 contains chicken manure compost and seaweed meal (plus Cu 0.01%, Fe 0.1%, Mn 0.04%, Zn 0.02%).
- Org 3: Organic fertilizer 5–3–16 is a chicken manure compost.
- Org 4: Organic fertilizer 9–1–4 is maltose based organic fertilizer (plus Mg 0.3%, S 3.0%, B 0.015%, Cu 0.1%, Fe 0.1%, Mn 0.7%, Mo 0.01%, Zn 0.01%).

### 2.2. Soil and Plant Description

The soil of the experimental area belongs to the soil subgroup Fibri–Dystric Histosol [25], with a peat layer 1.0–1.5 m deep and a flat field area. According to the classification of Estonian vegetation site types [26], this area is classified as an Oligotrophic (ombrotrophic), heavily drained bog of the raised bog site type; until the 1980s, it was used for industrial peat production—peat milling specifically. Soil samples were taken separately at 0 to 20 cm depth from each treatment replicate in the first week of August in 2013 and in 2014 (before the first harvest). To determine nutrient (P, K, Ca, Mg) concentration, pH<sub>KCl</sub> and organic matter content, five soil samples were collected from each plot, then air-dried and analyzed at the Plant Biochemistry Laboratory of the Estonian University of Life Sciences. The contents of available P, K, Ca and Mg were determined by the aluminium lactate method [27]. The mean nutrient contents of the experimental plots in 2013 and 2014 are shown in Table 1. Soil pH<sub>KCl</sub> was 3.2–3.6 and organic matter content ranged from 80% to 83% in 2013. Soil pH<sub>KCl</sub> was not analyzed in 2014—the organic matter content ranged from 82% to 83%.

**Table 1.** Nutrient (mg kg<sup>-1</sup>) and organic matter (%) content of the experimental treatments in 2013 and 2014.

Year	Treatment	pH	P	K	Ca	Mg	Org.
2013	Min	3.2	258	1530	1924	958	82
	Org 1	3.5	83	1280	2732	843	83
	Org 2	3.5	103	222	2630	812	82
	Org 3	3.6	56	151	2978	777	82
	Org 4	3.6	112	234	2720	799	80
2014	Min	<i>n.a.</i>	447	1680	2546	761	83
	Org 1	<i>n.a.</i>	87	1723	4677	743	82
	Org 2	<i>n.a.</i>	83	254	4378	690	83
	Org 3	<i>n.a.</i>	74	172	4291	661	83
	Org 4	<i>n.a.</i>	37	216	4649	677	82

Org. = organic matter%; *n.a.* = not analyzed.

For the leaf tissue analysis, 150 uniform, uninjured and fully expanded mature leaves were hand-picked in August 2013 and 2014 from each treatment replicate. Analysis were performed in the Plant Biochemistry Laboratory of the Estonian University of Life Sciences, and results are given in g 100 g<sup>-1</sup> of dry matter (DM). Based on values recommended by Hart et al. [28], N content in blueberry leaves was deficient in all treatments, and P, Ca and Mg content was optimal in all treatments; K was optimal in the Min in both years and in Org 1 in 2013, and deficient/near optimal in other treatments (Table 2).

**Table 2.** Nutrient content of the half-highbush blueberry cv. 'Northblue' leaves (g 100 g<sup>-1</sup> DM) in 2013 and 2014 depending on the fertilizer compared to recommended tissue nutrient contents.

Year	Treatment	N	P	K	Ca	Mg
2013	Min	1.27	0.13	0.50	0.46	0.19
	Org 1	1.24	0.09	0.42	0.48	0.19
	Org 2	1.42	0.10	0.37	0.59	0.26
	Org 3	1.34	0.08	0.32	0.60	0.22
	Org 4	1.33	0.08	0.36	0.53	0.19
2014	Min	1.18	0.13	0.47	0.43	0.17
	Org 1	1.18	0.09	0.37	0.51	0.17
	Org 2	1.19	0.10	0.31	0.62	0.19
	Org 3	1.18	0.09	0.28	0.68	0.19
	Org 4	1.20	0.08	0.34	0.55	0.20
Recommended levels		1.76–2.0	0.10–0.40	0.41–0.70	0.41–0.80	0.13–0.25

Recommended = Recommended tissue nutrient content% [28].

### 2.3. Weather Conditions

Estonia is situated in the northern part of Europe in the temperate climate zone, where the climate is between continental and maritime, with four seasons. The country is located in a humid zone, where the mean annual precipitation is 672 mm [29]. The vegetation period in Estonia is from May to September with a mean temperature of 13.4 °C. Based on long term (1981–2010) meteorological data, the mean annual temperature is 6.0 °C. The coldest month of the year is February with a mean temperature of −4.5 °C. The warmest month of the year is July with a mean temperature of 17.4 °C. Mean sunshine duration is 1766 h, where December has mean sunshine hours of 20.7 h (lowest) and July of 288 h (highest). Snow cover is usually from the middle of December to the end of March. Based on Estonian Weather Service reports [29], monthly (May–September) temperatures, precipitation and total sunshine hours of the experimental area from 2011–2015 (except 2014) compared to the mean of 1981–2010 are shown in the Table 3. In 2011, the temperatures were slightly higher compared to the 30-year mean values, especially in June and July (17.7 and 20.5, respectively), and had less precipitation.

2013 was also slightly warmer compared to the 30-year mean values and had less precipitation and more sunshine hours from June to September. In 2012, there was more precipitation in May and August—76 and 103 mm, respectively. In 2015, there was less precipitation in June, July and August; however, there were more sunshine hours in August compared to the 30-years mean.

**Table 3.** Mean monthly temperatures, precipitation and total duration of sunshine hours from 2011–2013 and 2015 compared to the mean of thirty years (1981–2010).

Year	Month	Temp. (°C)	Precip. (mm)	Sun. (h)	1981–2010		
					Temp. (°C)	Precip. (mm)	Sun. (h)
2011	May	11.6	47	280	11.5	55	257
	June	17.7	38	318	15.0	84	251
	July	20.5	59	262	17.6	72	269
	August	16.6	61	216	16.2	86	220
	September	12.9	61	154	11.0	61	136
2012	May	12.0	76	271	11.5	55	257
	June	13.8	89	252	15.0	84	251
	July	18.3	69	281	17.6	72	269
	August	15.2	103	171	16.2	86	220
	September	12.4	57	127	11.0	61	136
2013	May	14.9	73	286	11.5	55	257
	June	18.2	35	269	15.0	84	251
	July	17.9	59	272	17.6	72	269
	August	17.2	79	253	16.2	86	220
	September	11.3	23	186	11.0	61	136
2015	May	10.6	61	224	11.5	55	257
	June	14.6	66	251	15.0	84	25
	July	16.1	68	215	17.6	72	269
	August	17.0	47	305	16.2	86	220
	September	12.8	67	133	11.0	61	136

Temp. (°C) = temperature (°C); Precip. (mm) = precipitation (mm); Sun. (h) = total duration of sunshine hours (h).

#### 2.4. Determination of Vegetative and Yield Parameters

For bush height and diameter (cm) measurements, plants were measured from each treatment replicate with a ruler each year when the vegetative growth of the plants was complete. For bush height, the plants were measured from the base of the plant (soil level) to the top of the plant (highest point). For bush diameter, the plants were measured across the line, within the line and diagonally; from those measurements, the mean bush diameter was calculated.

For the berry mass (g) measurements, uniform and uninjured berries from the first harvest were weighed with scale Scaltec SAC 51. Berry mass was calculated as a mean of 30 fresh fruits from each treatment replicate. For the yield calculation ( $\text{g plant}^{-1}$ ), each year plants were hand-harvested three times or until all berries were ripened, with ca. one-week intervals. The criteria determining the stage of maturity was the fruit's full blue coloration. The yield of each bush from each harvest was weighed with a scale.

#### 2.5. Fruit Biochemical Analysis

Blueberries were harvested in the first weeks of August in every experimental year. Analysis were conducted from the first harvest. For preparing the laboratory samples, 250 g of berries were taken from each of the treatment replicates and pureed. Analysis were conducted on fresh berries one day after harvesting and expressed by fresh weight (FW). All the analysis and measurements were performed in three replicates.

The total phenol content (TPC) was determined using the Folin–Ciocalteu method [30] with a Shimadzu UV Visible Spectrophotometer UVmini-1240. Ethanol-acetone (7:3) solution was used as the

solvent to extract the phenolic compounds. In the experiment, 0.3 mL of the plant extract was mixed with 7.7 mL of distilled water and 0.5 mL of Folin–Ciocalteu reagent (1:1 solution with water). After 1 min, 1.5 mL of a 2% sodium carbonate solution was added. Samples were held for 2 h at 22 °C and the absorbance was read at 765 nm. The total phenol content was expressed as mg of gallic acid per 100 g of fresh berries.

The total anthocyanin content (ACC) was estimated using the pH differential method [30]. Absorbance was measured with a Shimadzu UV Visible Spectrophotometer UVmini-1240 at 510 nm and at 700 nm in buffers at pH 1.0 (HCl 0.1 N) and pH 4.5 (citrate buffer). The extraction solution contained hydrochloric acid (0.1 M) and ethanol (96%) in *v:v* ratio of 15:85. The results were expressed as mg of cyaniding–3–glycoside per 100 g of fresh berries.

A Pocket Pal–1 digital hand-held refractometer (Atago) was used for soluble solids content (SSC) measurements. SSC was estimated as °Brix. Titratable acid content (TAC) was analyzed using a standard acid–base titration method [30]. An aliquot of sample (40 mL) was titrated with 0.1 M NaOH solution to a phenolphthalein endpoint (pH 8.2). EasyPlus Trtitration (Mettler Toledo) was used for measuring (with electrode DG 111-SC for endpoint detections). Titratable acids content was expressed as mg citric acid per 100 g of fruit fresh weight (FW), as citric acid was the dominant organic acid in blueberries, using the milliequivalent factor of 0.064 for the citric acid. The Brix/acid ratio (SSC/TAC) was calculated by dividing soluble solids by titratable acids content. For the determination of ascorbic acid content (ASC), hydrochloric and acetic acids were immediately added to the fruit puree to avoid ascorbic acid breakdown in the air. ASC was titrated with the solution of 2,6–dichlorophenolindophenol [31] using an automatic titrator (EasyPlus, Mettler-Toledo International Inc.), and expressed as mg 100 g<sup>−1</sup> FW.

## 2.6. Statistical Analysis

All measurements were carried out on three parallel samples for each variable and data were expressed in tables as the mean value ± standard deviation (SD). The data were evaluated by one–way analysis of variance (ANOVA), and the means were compared using a Fisher’s least significant difference (LSD) test at a 5% probability level. A principal component analysis (PCA) was performed to describe the structure of all the analyzed parameters in relation to the fertilizers and experimental years. A principal component analysis (PCA) was applied to describe the structure of all the analyzed parameters in relation to the fertilizers and experimental years. The PCA is a dimensional modelling method that helps to visualize correlations between data points and give an interpretable overview of the main information from multidimensional data, in which the results are estimated and summarized into a few underlying variables. Analyses were performed using standardized mean data. All analyses were performed using Statistica for Windows version 12.0 (StatSoft, Inc., Tulsa, OK, USA).

## 3. Results

### 3.1. Vegetative and Yield Parameters

Bush diameter ranged from 75 to 118 cm in the experiment (Table 4). Fertilizers did not have an effect on bush diameter or height in 2013 and 2015. In 2011, the bush diameter was statistically smaller with the use of Org 4 fertilizer compared to with the other treatments. In 2012, a similar trend continued, where the Org 4 treatment resulted in a smaller bush diameter compared to the Org 1 and Org 2 treatments. The use of the Org 4 treatment also resulted in lower bush height in 2011 and 2012.

**Table 4.** The effect of fertilizing on the half-highbush blueberry cv. ‘Northblue’ berry mass, bush diameter, bush height and yield in 2011–2013 and 2015.

Year	Treatment	Bush Diameter	Bush Height	Berry Mass	Yield
2011	Min	100 ± 9 <sup>a</sup>	83 ± 4 <sup>a</sup>	2.3 ± 0.2 <sup>a</sup>	568 ± 8 <sup>d</sup>
	Org 1	94 ± 4 <sup>a</sup>	78 ± 5 <sup>a</sup>	2.1 ± 0.3 <sup>a</sup>	412 ± 32 <sup>e</sup>
	Org 2	96 ± 5 <sup>a</sup>	82 ± 7 <sup>a</sup>	2.1 ± 0.1 <sup>a</sup>	1222 ± 33 <sup>a</sup>
	Org 3	96 ± 6 <sup>a</sup>	82 ± 4 <sup>a</sup>	2.3 ± 0.1 <sup>a</sup>	878 ± 13 <sup>b</sup>
	Org 4	75 ± 8 <sup>b</sup>	59 ± 3 <sup>b</sup>	2.0 ± 0.1 <sup>a</sup>	827 ± 28 <sup>c</sup>
2012	Min	109 ± 3 <sup>a,b</sup>	81 ± 1 <sup>a</sup>	2.5 ± 0.1 <sup>a,b</sup>	2043 ± 41 <sup>a</sup>
	Org 1	116 ± 3 <sup>a</sup>	82 ± 1.8 <sup>a</sup>	2.7 ± 0.1 <sup>a</sup>	1986 ± 78 <sup>a</sup>
	Org 2	112 ± 11 <sup>a</sup>	79 ± 2 <sup>a,b</sup>	2.7 ± 0.2 <sup>a</sup>	2014 ± 59 <sup>a</sup>
	Org 3	107 ± 5 <sup>a,b</sup>	77 ± 4 <sup>b,c</sup>	2.4 ± 0.2 <sup>b</sup>	1508 ± 62 <sup>b</sup>
	Org 4	99 ± 2 <sup>b</sup>	73 ± 1 <sup>c</sup>	2.7 ± 0.2 <sup>a</sup>	1126 ± 82 <sup>c</sup>
2013	Min	103 ± 10 <sup>a</sup>	87 ± 7 <sup>a</sup>	1.6 ± 0.4 <sup>a</sup>	328 ± 28 <sup>c</sup>
	Org 1	100 ± 8 <sup>a</sup>	85 ± 8 <sup>a</sup>	1.4 ± 0.1 <sup>a</sup>	335 ± 38 <sup>c</sup>
	Org 2	101 ± 4 <sup>a</sup>	89 ± 6 <sup>a</sup>	1.3 ± 0.0 <sup>a</sup>	649 ± 13 <sup>a</sup>
	Org 3	96 ± 6 <sup>a</sup>	86 ± 6 <sup>a</sup>	1.4 ± 0.1 <sup>a</sup>	285 ± 38 <sup>c</sup>
	Org 4	93 ± 3 <sup>a</sup>	78 ± 6 <sup>a</sup>	1.4 ± 0.1 <sup>a</sup>	579 ± 42 <sup>b</sup>
2015	Min	118 ± 10 <sup>a</sup>	100 ± 4 <sup>a</sup>	1.9 ± 0.1 <sup>a</sup>	1046 ± 360 <sup>a</sup>
	Org 1	117 ± 4 <sup>a</sup>	105 ± 5 <sup>a</sup>	1.9 ± 0.1 <sup>a,b</sup>	796 ± 29 <sup>a</sup>
	Org 2	111 ± 9 <sup>a</sup>	100 ± 7 <sup>a</sup>	1.7 ± 0.1 <sup>b</sup>	859 ± 309 <sup>a</sup>
	Org 3	108 ± 3 <sup>a</sup>	98 ± 4 <sup>a</sup>	2.0 ± 0.1 <sup>a</sup>	723 ± 110 <sup>a</sup>
	Org 4	109 ± 3 <sup>a</sup>	100 ± 2 <sup>a</sup>	1.7 ± 0.2 <sup>b</sup>	741 ± 143 <sup>a</sup>

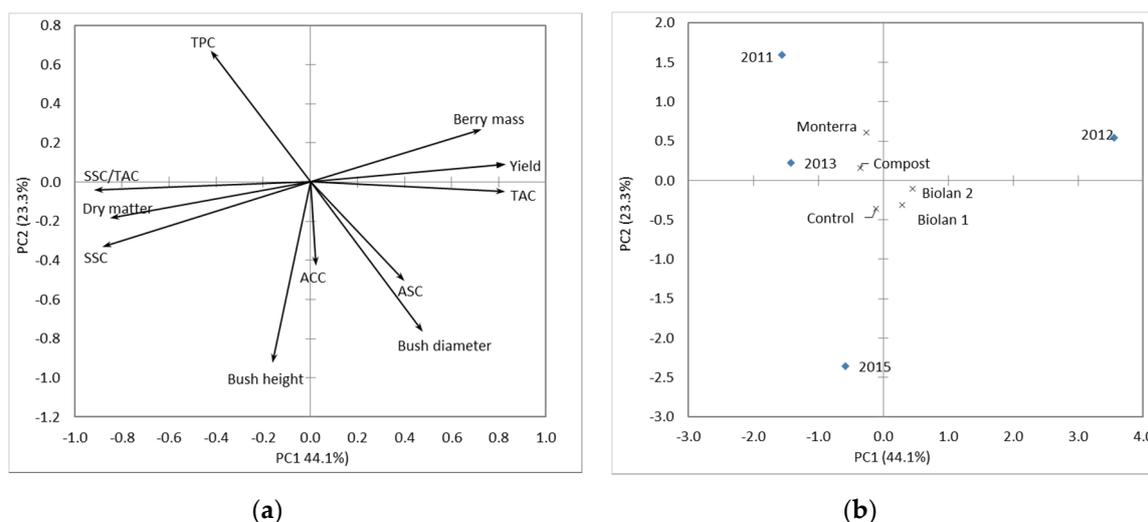
Bush diameter (cm); bush height (cm); yield (g plant<sup>-1</sup>); berry mass (g); ± SD. Means for each parameter followed by the different letter within each year in each column are significantly different ( $p \leq 0.05$ ).

Berry mass did not differ significantly in 2011 and 2013 between the treatment types—ranging from 2.0 to 2.3 g in 2011, and 1.3–1.6 g in 2013 (Table 4). In 2012 and 2015, there were statistical differences, but no obvious trend was noticed. In 2012, the use of the Org 1, Org 2 and Org 4 fertilizers had a positive effect on berry mass compared to the use of Org 3. In 2015, the use of Min and Org 3 had a positive effect on berry mass compared to the results for the use of Org 2 and Org 4.

The fertilizers had a significant effect on the yield, which ranged from 285 to 2043 g plant<sup>-1</sup> (Table 4). The use of the Org 2 treatment resulted the highest yield in 2011 and 2013. In 2011, the yield from the Org 2 treatment was more than double than those of Min and Org 1 treatments, and more than one third higher than for those of Org 3 and Org 4 treatments. In 2012, a higher yield was obtained from the Min, Org 2 and Org 1 treatments compared to that of the Org 3 and Org 4 treatments. In 2015, the yields ranged from 723 to 1046 g plant<sup>-1</sup>, and no effect of fertilizing was observed.

### 3.2. Effect of the Fertilizer and Experimental Year on the Biochemical and Vegetative Parameters of Blueberries as Characterized on PCA

The PCA showed that the first principal component (PC1) explained 44% of the total variance in the data, and the second principal component (PC2) explained 23% (Figure 1). The PC1 and PC2 explained 68% of the variance in the data for both Figure 1a,b. PC3 explained 12% of the variance in the data in Figure 1a and described the negative correlation of ACC (data not presented in figure). PC1 was more related to the SSC, TAC, SSC/TAC, berry mass, dry matter and yield, whereas total PC2 was more related to the TPC, bush height and bush diameter in Figure 1a. TPC was positively correlated with 2011 in PC2; while yield, berry mass and TAC were positively correlated in PC1 and were highest in 2012.



**Figure 1.** Principal component analysis (PCA) of the structure of biochemical and vegetative parameters in relation to the fertilizer type and experimental year's climate: (a) biochemical parameters in relation to the vegetative parameters; (b) fertilizer type in relation to the experimental year.

PCA demonstrated that experimental years distinguished more clearly than different fertilizers, and that their relative importance in determining fruit characteristics was larger (Figure 1). For instance, high yield and large fruits were characteristic of 2012, and high total polyphenol content was characteristic of 2011. The PCA also showed a close positive relationship between the berry mass and TAC of fruits and negative relationship between berry mass and SSC of the fruit.

### 3.3. Fruit Biochemical Parameters

The TPC ranged from 134 to 220 mg 100 g<sup>-1</sup> FW, and the effect of the fertilizer was significant (Table 5). Fruits produced under the Min treatment had a higher TPC in 2012, 2013 and 2015 compared to those produced under the Org 1 treatment. The Org 2 treatment resulted in statistically lower fruit TPC in 2012 and in 2015 compared to that of the fruits produced under the Min treatment. The ACC ranged from 48 to 136 mg 100 g<sup>-1</sup> FW. The use of Min resulted in statistically lower ACC in fruits in 2012 and in 2013 compared to the use of the Org 4 treatment. Treatment with Org 4 resulted in higher ACC in 2012, 2013 and 2015 compared to treatment with Org 1.

The SSC ranged from 9.6 to 11.9 Brix° in the experiment, with a significant effect of the fertilizers being observed (Table 5). In 2011, the SSC was statistically lower with the use of the Org 1 and Org 2 fertilizers, where Org 4 treatment resulted in the highest SSC. The Min treatment resulted in the highest SSC in 2012 compared to the Org 2 and Org 4 treatments. In 2013, fertilizing with Org 1 and Org 2 resulted in the lowest SSC compared to the other treatments. In 2015, a similar trend continued, where Org 1 resulted in the lowest SSC compared to the Org 3 and Org 4 treatments. TAC did not have any statistical differences between the treatments in 2011, 2013 and 2015; however, in 2012, the TAC was statistically different with the use of Min fertilizer compared to the organic fertilizers. SSC/TAC varied between the treatments in 2012, where the highest SSC/TAC was achieved with Min fertilizer.

ASC had high variance in the experiment, ranging from 4.5 to 18.3 g 100 g<sup>-1</sup> FW (Table 5). In 2011, the Org 4 treatment resulted in statistically higher ASC compared to the other treatments, where the lowest results were obtained with the use of Org 2. In 2012, there was an opposite effect, where the Org 4 treatment resulted in relatively low ASC compared to the Min treatment. In 2013, a similar trend continued, where Min treatment resulted in higher ASC compared to the Org 4 treatment, and to the other organic fertilizers' ASC. In 2015, Min resulted in a higher ASC compared to the Org 4 treatment.

**Table 5.** The effect of the fertilizer on the half-highbush blueberry cv. ‘Northblue’ biochemical parameters in 2011–2013 and 2015.

Year	Treatment	TPC	ACC	SSC	TAC	SSC/TAC	ASC
2011	Min	207 ± 10 <sup>a,b</sup>	113 ± 7 <sup>a</sup>	11.2 ± 0.1 <sup>b</sup>	0.8 ± 0.1 <sup>a</sup>	14.4 ± 1.5 <sup>a</sup>	9.5 ± 0.4 <sup>b</sup>
	Org 1	195 ± 10 <sup>b,c</sup>	97 ± 6 <sup>b</sup>	10.8 ± 0.1 <sup>c</sup>	0.8 ± 0.1 <sup>a</sup>	13.6 ± 1.6 <sup>a</sup>	6.6 ± 0.4 <sup>c</sup>
	Org 2	206 ± 10 <sup>a,b,c</sup>	113 ± 12 <sup>a</sup>	10.8 ± 0.1 <sup>c</sup>	0.8 ± 0.1 <sup>a</sup>	13.6 ± 1.4 <sup>a</sup>	4.5 ± 0.1 <sup>d</sup>
	Org 3	191 ± 5 <sup>c</sup>	100 ± 11 <sup>a,b</sup>	11.2 ± 0.1 <sup>b</sup>	0.7 ± 0.1 <sup>a</sup>	16.2 ± 2.3 <sup>a</sup>	6.3 ± 0.2 <sup>c</sup>
	Org 4	220 ± 10 <sup>a</sup>	78 ± 4 <sup>c</sup>	11.7 ± 0.1 <sup>a</sup>	0.8 ± 0.1 <sup>a</sup>	14.7 ± 1.3 <sup>a</sup>	10.4 ± 0.8 <sup>a</sup>
2012	Min	182 ± 10 <sup>a</sup>	75 ± 8 <sup>c</sup>	9.9 ± 0.1 <sup>a</sup>	0.9 ± 0.1 <sup>b</sup>	11.1 ± 0.7 <sup>a</sup>	18.3 ± 0.7 <sup>a</sup>
	Org 1	147 ± 10 <sup>b</sup>	65 ± 7 <sup>c</sup>	9.6 ± 0.1 <sup>b</sup>	1.1 ± 0.1 <sup>a</sup>	8.7 ± 0.6 <sup>b</sup>	13.5 ± 0.2 <sup>c</sup>
	Org 2	148 ± 11 <sup>b</sup>	79 ± 4 <sup>c</sup>	9.1 ± 0.1 <sup>c</sup>	1.1 ± 0.1 <sup>a</sup>	8.1 ± 0.6 <sup>b</sup>	14.5 ± 0.2 <sup>b</sup>
	Org 3	191 ± 13 <sup>a</sup>	103 ± 13 <sup>b</sup>	9.6 ± 0.1 <sup>b</sup>	1.1 ± 0.1 <sup>a</sup>	8.7 ± 0.7 <sup>b</sup>	12.5 ± 0.5 <sup>d</sup>
	Org 4	157 ± 10 <sup>b</sup>	130 ± 16 <sup>a</sup>	9.1 ± 0.1 <sup>c</sup>	1.1 ± 0.1 <sup>a</sup>	8.0 ± 0.7 <sup>b</sup>	13.0 ± 0.0 <sup>c,d</sup>
2013	Min	204 ± 19 <sup>a</sup>	60 ± 6 <sup>b</sup>	11.9 ± 0.1 <sup>a</sup>	0.8 ± 0.1 <sup>a</sup>	14.3 ± 2.0 <sup>a</sup>	16.4 ± 0.3 <sup>a</sup>
	Org 1	149 ± 17 <sup>b</sup>	48 ± 8 <sup>c</sup>	11.6 ± 0.1 <sup>b</sup>	0.9 ± 0.1 <sup>a</sup>	13.4 ± 1.4 <sup>a</sup>	11.1 ± 1.1 <sup>b</sup>
	Org 2	193 ± 20 <sup>a</sup>	73 ± 7 <sup>a</sup>	11.6 ± 0.1 <sup>b</sup>	0.9 ± 0.1 <sup>a</sup>	13.0 ± 1.5 <sup>a</sup>	8.3 ± 0.4 <sup>c</sup>
	Org 3	198 ± 10 <sup>a</sup>	58 ± 6 <sup>b,c</sup>	11.8 ± 0.1 <sup>a</sup>	0.9 ± 0.1 <sup>a</sup>	13.4 ± 1.3 <sup>a</sup>	12.6 ± 1.7 <sup>b</sup>
	Org 4	194 ± 11 <sup>a</sup>	75 ± 5 <sup>a</sup>	11.9 ± 0.2 <sup>a</sup>	0.9 ± 0.1 <sup>a</sup>	12.9 ± 1.2 <sup>a</sup>	13.2 ± 1.7 <sup>b</sup>
2015	Min	170 ± 5 <sup>a</sup>	136 ± 8 <sup>a</sup>	11.8 ± 0.1 <sup>a,b</sup>	0.9 ± 0.1 <sup>a</sup>	13.3 ± 0.9 <sup>a</sup>	15.5 ± 1.0 <sup>a</sup>
	Org 1	146 ± 5 <sup>b,c</sup>	108 ± 16 <sup>b</sup>	11.6 ± 0.1 <sup>b</sup>	0.9 ± 0.1 <sup>a</sup>	13.4 ± 0.9 <sup>a</sup>	14.4 ± 1.0 <sup>a,b</sup>
	Org 2	134 ± 10 <sup>c</sup>	131 ± 5 <sup>a</sup>	11.7 ± 0.1 <sup>a,b</sup>	0.9 ± 0.1 <sup>a</sup>	13.3 ± 0.6 <sup>a</sup>	14.1 ± 0.8 <sup>a,b</sup>
	Org 3	158 ± 10 <sup>a,b</sup>	116 ± 12 <sup>a,b</sup>	11.9 ± 0.2 <sup>a</sup>	0.9 ± 0.1 <sup>a</sup>	13.7 ± 1.1 <sup>a</sup>	14.8 ± 0.8 <sup>a</sup>
	Org 4	158 ± 10 <sup>a,b</sup>	135 ± 10 <sup>a</sup>	11.8 ± 0.1 <sup>a</sup>	0.9 ± 0.1 <sup>a</sup>	13.0 ± 1.2 <sup>a</sup>	13.3 ± 0.3 <sup>b</sup>

TPC = total phenol content (mg 100 g<sup>-1</sup> FW); ACC = total anthocyanin content (mg 100 g<sup>-1</sup> FW); SSC = soluble solids content (Brix°); TAC = titratable acid content (g 100 g<sup>-1</sup> FW); SSC/TA = soluble solids and titratable acid content ratio; ASC = ascorbic acid content (mg 100 g<sup>-1</sup> FW); ±SD. Means for each parameter followed by the different letter within each year in each column are significantly different ( $p \leq 0.05$ ).

## 4. Discussion

### 4.1. Vegetative Parameters

Blueberry plants had reached the mature stage in the experiment. In the first two years, the bush diameter and height were smaller with the use of Org 4; however, growth stabilized by the end of the experiment, where different treatment types did not have a significant effect on the vegetative parameters. Growth parameters in our study were affected by winter damage, and due to that, the results were fluctuating; however, the plants reached a height specific to their species. An earlier study stated that blueberries are more susceptible to winter damage on peat soil compared to mineral soil, although in peat soil the cv. ‘Northblue’ performed better than the other half-highbush cultivars [12]. Bush height varied in 2011 from 59 cm to 83 cm, and increased from 98 to 105 cm by the end of the experiment. In a previously mentioned study conducted on peat soil with cv. ‘Northblue’, the plant height was 48 cm, which was lower than in our study [12].

Although fertilizer had a significant effect on yield, the experimental year had a stronger impact, resulting in over sevenfold differences in yield. Yearly differences were caused by weather conditions. The effect of the experimental year was demonstrated by PCA, showing that the experimental year’s weather had a strong effect on fruit parameters, while the fertilizer type did not have a strong correlation with the variables. Previous studies have suggested that different environmental conditions affect blueberry mineral nutrition [31], but so do the year, site and cultivar interaction [12]. Once the plants reach full maturity, the yield fluctuates from year to year as a result of weather conditions and pruning [16]. As the abandoned peatlands in Estonia have similar organic matter content and soil acidity, the field to field variation of these areas is more modest than on mineral soil, and the yearly weather has a more significant impact on the fertilizer’s effects on yield [12,17]. Previous studies on abandoned peatlands showed that blueberry cultivation without fertilizer produces yields that are near negligible; therefore, fertilization is necessary to provide adequate productivity [9]. In our

study, in 2011 and 2013, the temperatures were slightly higher and there were less precipitation and more sunshine hours from June to September in 2013 compared to the 30-year mean values. In both years, the highest yield was achieved with the Org 2 treatment. However, in 2013, the yield was rather low compared to other experimental years. An earlier study conducted with lowbush blueberry on peat soil also concluded that fertilization had an effect and high variance on yield where yield varied from 14 to 393 g plant<sup>-1</sup> in a 5-year-old plantation; however, the effect of the fertilizer was weather-dependent [9]. Despite of the weather conditions in our study, the use of Org 2 fertilizer gave a stable yield performance in many experimental years. All of the fertilizers used in our experiment were granulated, but differed in composition; the Org 1, Org 2 and Org 3 contained chicken manure, and the Org 4 contained maltose, but also differed in phosphorus and potassium content. With reference to the different fertilizer compositions, the timescale of fertilizer decomposition may also have been different, as well as weather dependent. In 2011, there was less precipitation from May to August, which may have had a negative effect on nutrient uptake and plant growth.

Organic fertilizers used in the study showed positive results compared to the mineral fertilizer. Although organic fertilizer Org 2 had lower potassium and phosphorus content compared to the mineral fertilizer, it resulted in similar vegetative growth and a high yield. As described, the Org 2 fertilizer contains seaweed. Many studies have stated a wide range of beneficial effects of seaweed extract application on plants, such as early seed germination and establishment, improved crop performance and yield, increased resistance to biotic and abiotic stress, and the improved postharvest shelf-life of perishable commodities [32–35]. Seaweed contains macro- and micro-elements, amino acids, vitamins and growth hormones like cytokinins, auxins, and abscisic acid, that have an effect on cellular metabolism in plants resulting in improved growth and yield [36–38]. Seaweed also affects the chemical, physical and biological properties of soil, which all influence plant growth. Its extracts improve soil health by enhancing the moisture-holding capacity, increasing the growth of beneficial soil microbes, and encouraging the growth of beneficial fungi to stimulate mycorrhizal development [35,39]. A study found that seaweed oligosaccharides, which are produced by the enzymatic degradation of alginic acid, significantly improved the hyphal growth and elongation of arbuscular mycorrhizal fungi, but also activated their infectivity on trifoliolate orange seedlings [40]. Although the organic fertilizer Org 2 had a lower P and K content, the positive performance could be related to the seaweed concentration, which may have a beneficial effect on soil biota, mycorrhizal development and plant growth, although this needs further research.

#### 4.2. Biochemical Parameters

The biochemical composition was affected by the fertilizer type and the year. The use of the Min treatment had higher TPC in 2012, 2013 and 2015 compared to the organic fertilizer Org 1. Valuable antioxidants in blueberries include phenolic compounds, the major role of which is to protect organisms against the oxidative stress induced by free radicals [41]. Previous studies have stated that the organically grown blueberry cultivar ‘Powderblue’ had a higher TPC compared to conventionally grown cultivars [41]. Another study conducted with different high-bush cultivars compared TPC results from conventional and organic farms and found that there were no statistical differences between the cultivation types, except that there were significantly higher tannin levels in plants grown under organic cultivation [42]. Moreover, a study by Wang et al. [43] showed that blueberry fruit grown from organic culture yielded significantly higher TPC, malic acid, total anthocyanin content, antioxidant activity and sugars (fructose and glucose), than fruit from the conventional culture. Oppositely, in our study, the use of Min fertilizer increased TPC during some of the experimental years compared to some organic fertilizers. A study established in Korea with different half-highbush cultivars had a TPC of cv. ‘Northblue’ of 247.8 [44], which indicates species-specific TPC results from our experiment.

There was high variability in ACC caused by the treatments. The use of the organic fertilizer Org 4 resulted in higher ACC in most of the experimental years compared to Org 1 and Min. Cultivation practices are the main factors that affect the concentration of anthocyanins in fruits depending on the

genotype [15]. A study conducted by You et al. [41] found that the total anthocyanin content and total phenol content accumulated in different blueberry cultivars was either more or comparable in the case of organically grown cultivars compared to in conventionally grown rabbiteye blueberries. A similar study that focused on the effect of different cultivation practices on highbush blueberries, stated that the total anthocyanin content was significantly higher in organically cultivated blueberries [43].

ASC was more affected by the experimental year than by the fertilization method. A previous study with cv. 'Northblue' stated an ASC of ca. 15 mg 100 g<sup>-1</sup> FW [14], which is similar to the 2015 results. An earlier study concluded that climatic factors like light intensity and temperature are the most important in determining the final ASC [45]. It has been reported that the cooler climate and higher light intensity tend to increase the content of ASC [9]. In our study, the temperatures were slightly higher with less precipitation in June and July 2011 compared to the 30-year mean; however, the ASC was lower compared to other experimental years. Overall, the use on Min fertilizer tended to result in a higher ASC. In 2012 and 2015, the Min treatment resulted in a higher ASC in fruits compared to the Org 4. In 2013, the use of Min resulted in highest ASC compared to organic fertilizers.

The fertilizers used in our experiment had a different effect on biochemical composition due to the different composition. The Min fertilizer had higher potassium (19%) content compared to organic fertilizers. Previous studies suggest that adequate potassium nutrition greatly influences the synthesis of sucrose and starch in different fruits, berries and vegetables, for example in apple [46,47] and in strawberry [48]. Potassium levels have different effects on organic acid metabolism depending on the plant species [49] and also strengthen photosynthesis at the source to supply the sink with enough carbohydrates [50]. In an experiment with apples, K fertilization promoted higher berry mass, higher Ca<sup>2+</sup>, soluble solid content, and lower TAC [47]. In our study, the K content in the leaves was optimal with the use of mineral fertilizer and the Org 1. Org 1 fertilizer also had a higher K content in the fertilizer (7%) compared to the other fertilizers used in the study. According to Hart et al. [28] the optimum K range in blueberry leaves is 0.41%–0.70%. Despite the higher K content in the Min and Org 1 fertilizer, no clear trends in the effect on biochemical content were observed. Although, in some experimental years, the use of Min treatment resulted in higher TPC and ASC, decreased TAC and increased SSC/TAC content, but this effect was not observed in every experimental year.

## 5. Conclusions

As the abandoned peatlands are environmentally sensitive areas, we conclude that organic fertilizers are feasible alternatives to mineral fertilizers for half-highbush blueberry cv. 'Northblue' organic cultivation under abandoned peatland conditions; thus, the hypothesis was confirmed. Since blueberry producers are primarily interested in high yield, the low nutrient fertilizer Org 2 could be recommended for organic production in peatland areas due to its more stable yield performance. Based on results, it may be concluded that fertilizer composition had an effect on plant vegetative and fruit biochemical parameters; however, the experimental year had more significant impact.

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